



## Regulation of secondary metabolism in *Streptomyces coelicolor* A3(2)

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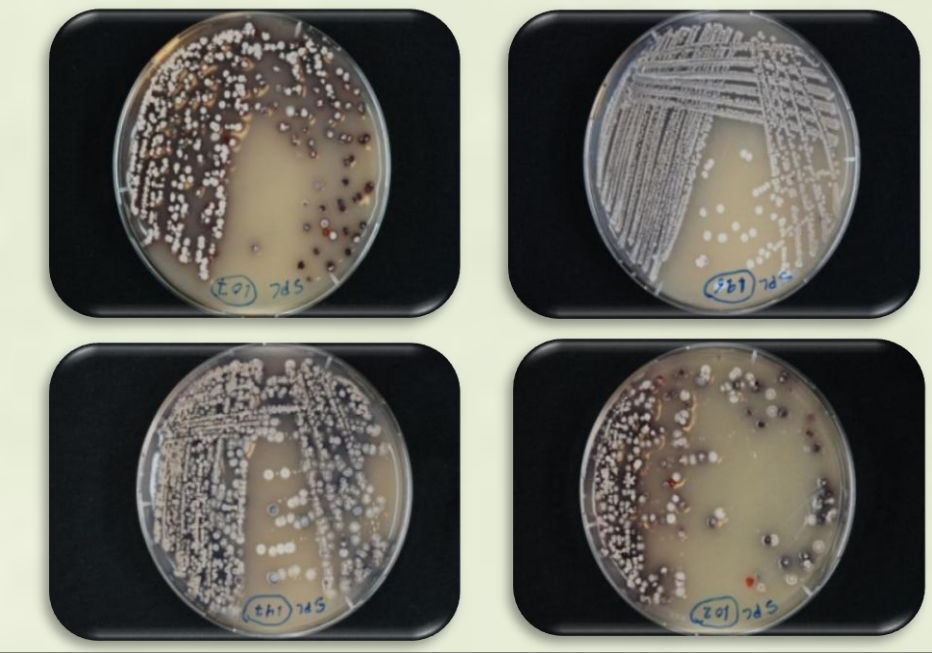
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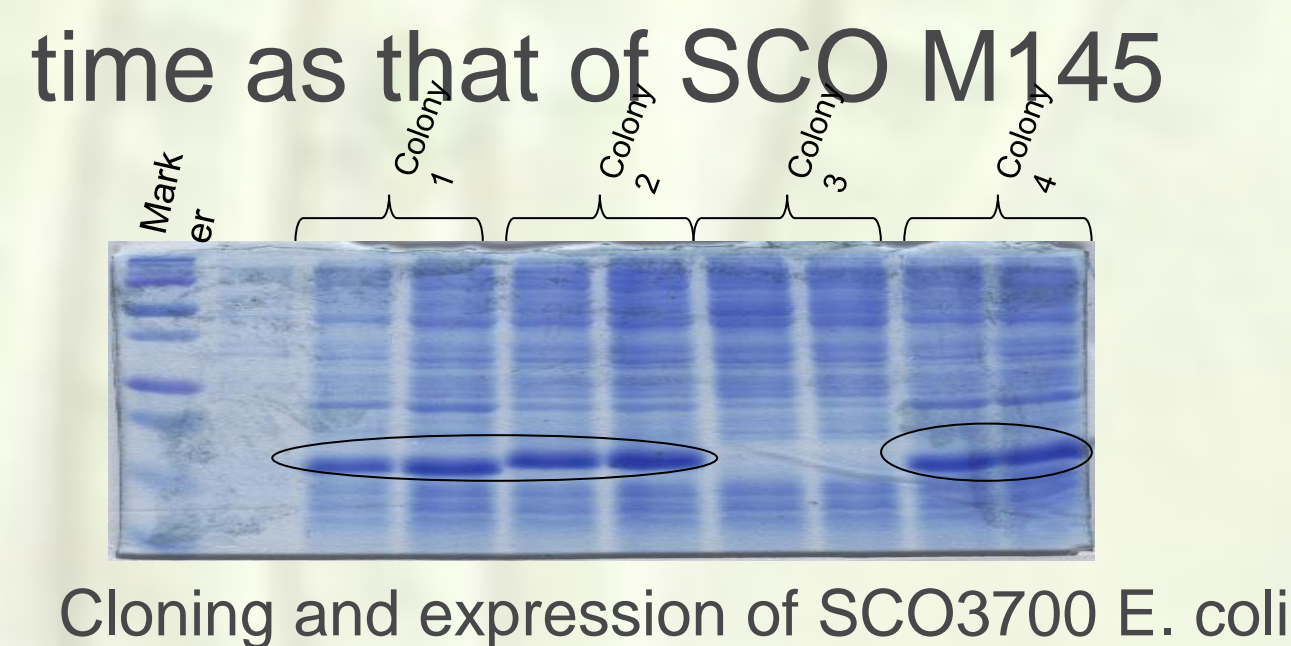
## Intro

- Three approaches were taken in order to study the regulation of actinorhodin;
- (1) investigation of the influence of protein tyrosine phosphatase on actinorhodin biosynthesis
  - (2) modification of regulation on actinorhodin cluster by randomizing the native *act II orf4* promoter and
  - (3) modification of the redox levels inside the cell

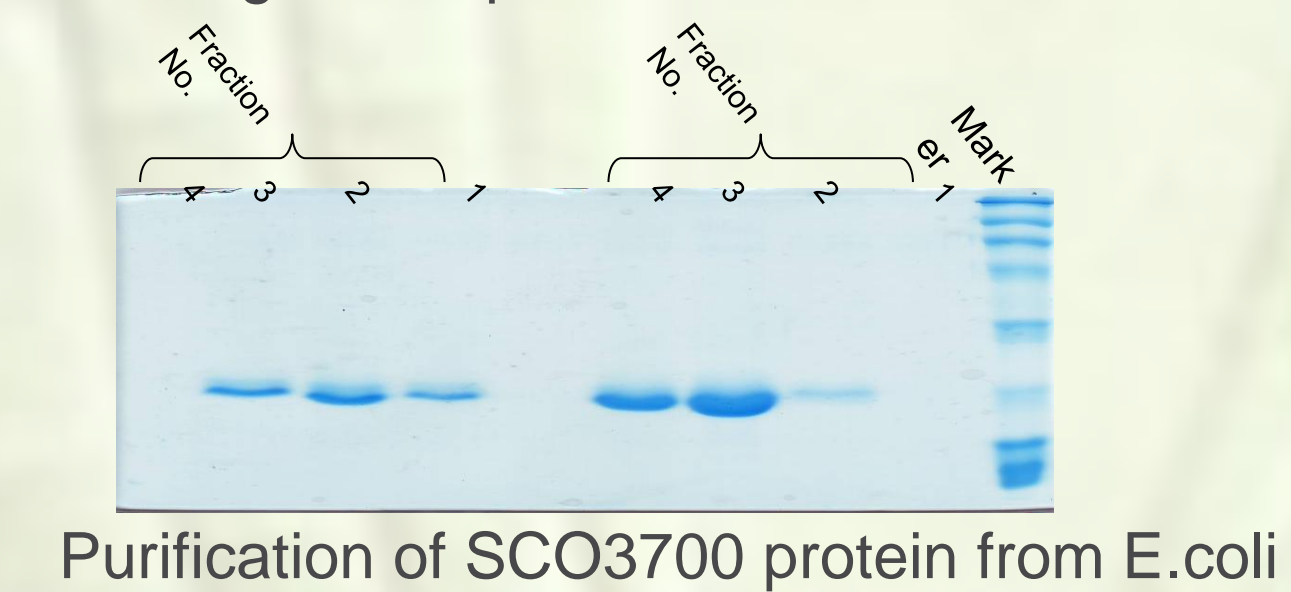


## SCO3700 is a tyrosine phosphatase

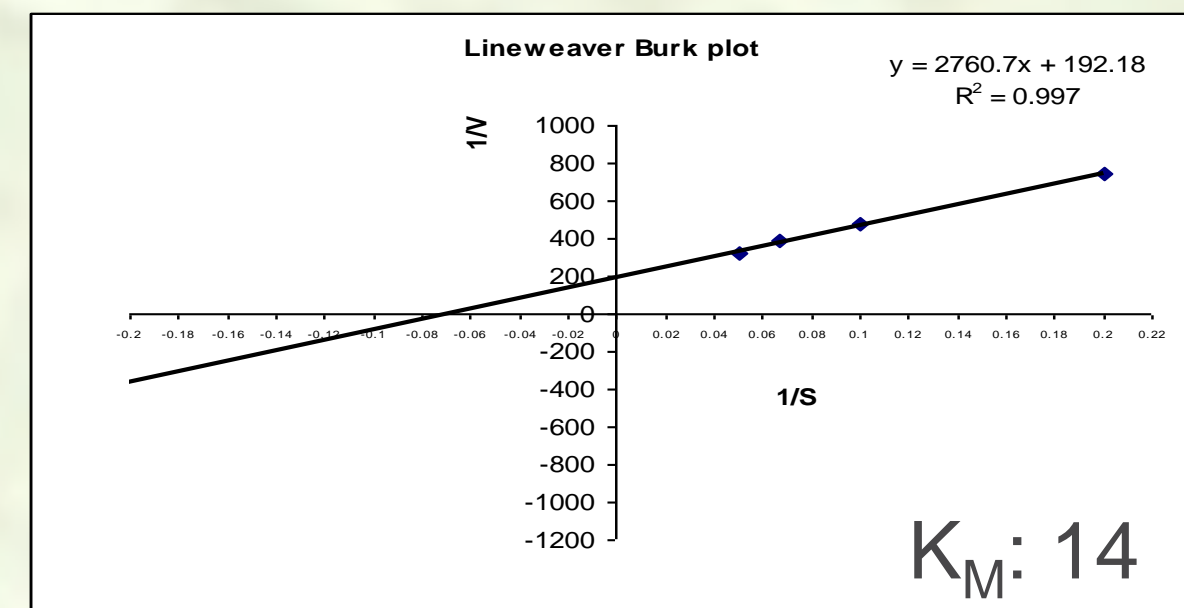
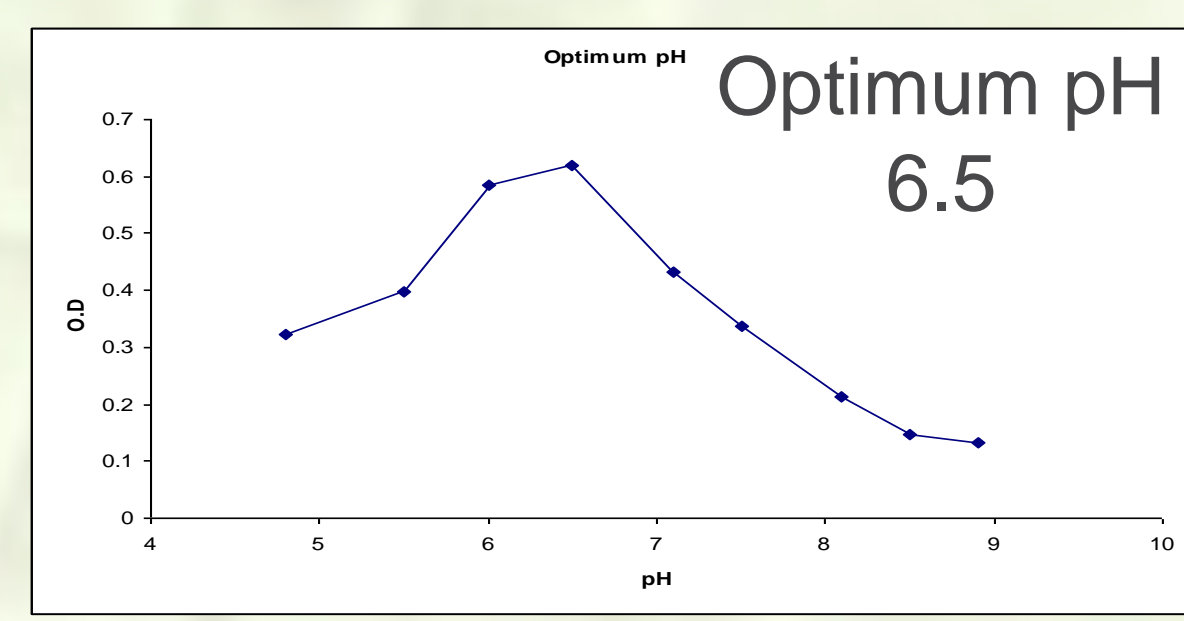
The genome of *Streptomyces coelicolor* contains 40 kinases and a few annotated phosphatases. Little is understood about their role in regulatory cascades and relation to secondary metabolite production. We have investigated the role of phosphotyrosine-protein phosphatases (PTPs). SCO3700 might be taking over the function of PtpA in SCO  $\Delta$ PtpA mutant. In SCOM145 oxp PtpA mutant onset of Antibiotic synthesis is early compared with wild type while in SCOM145  $\Delta$ PtpA mutant onset of UDP synthesis is delayed, while the Actinorhodin synthesis starts at around same time as that of SCO M145



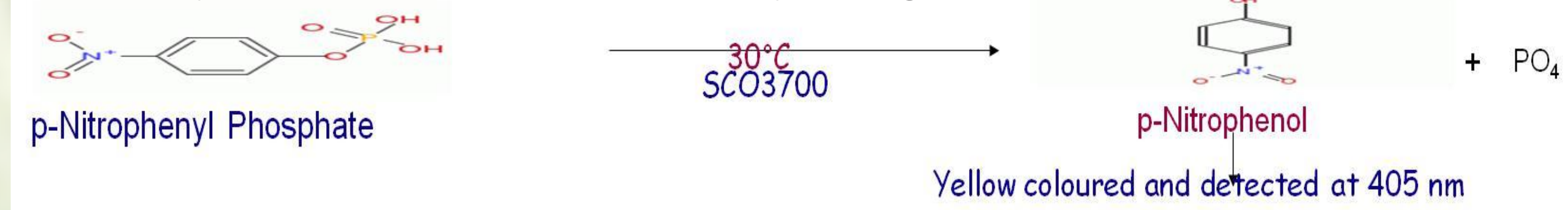
Cloning and expression of SCO3700 E. coli



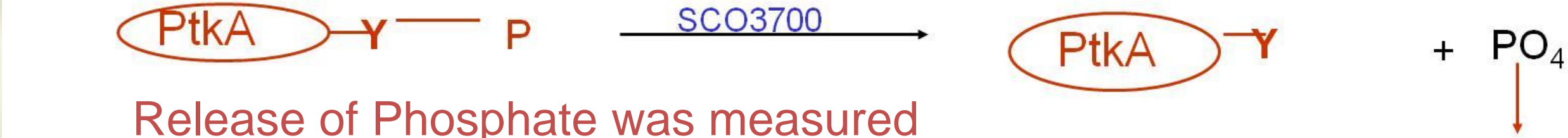
Purification of SCO3700 protein from E.coli



In vitro Tyrosine Phosphatase activity using PNPP as substrate

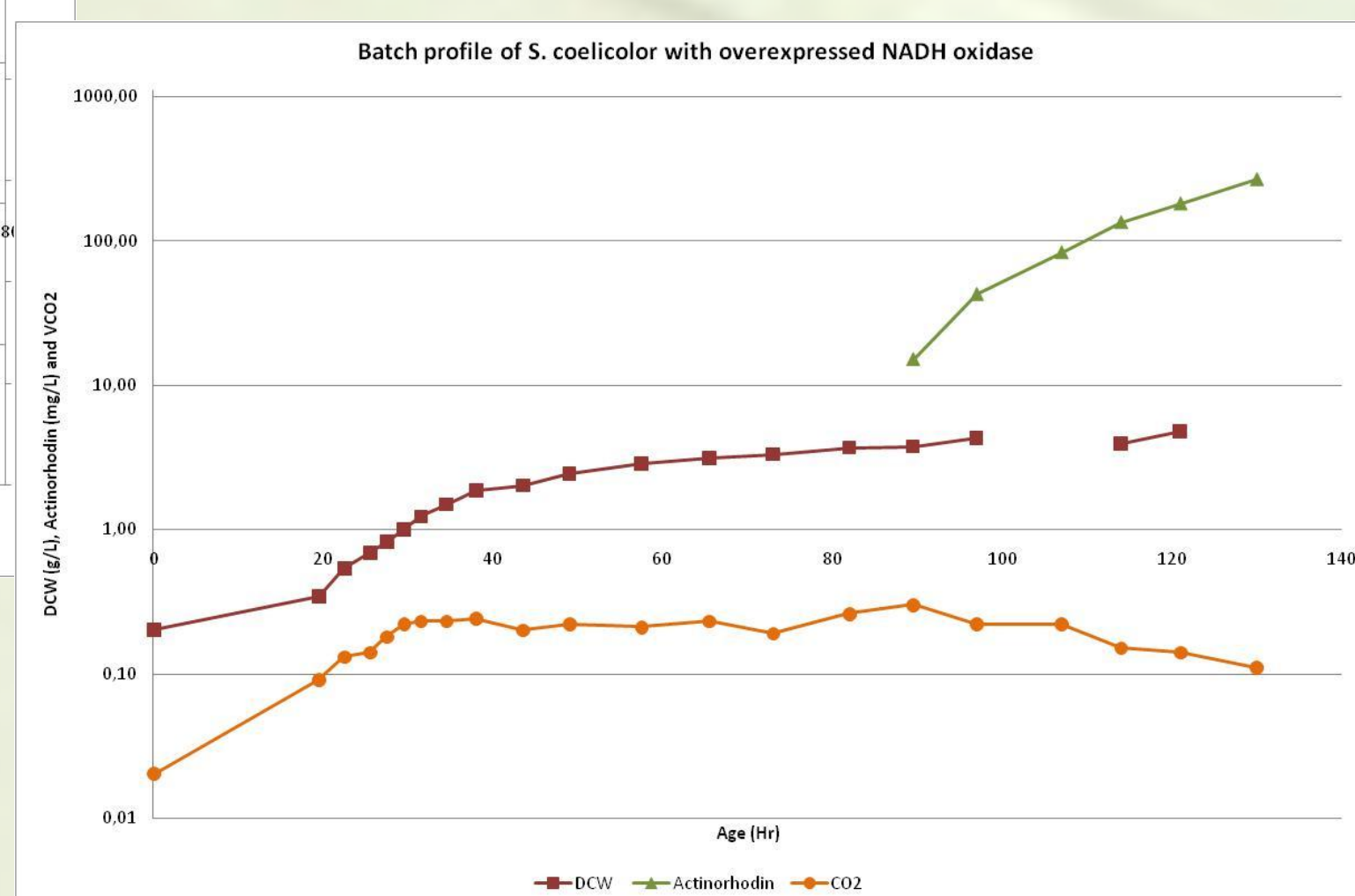
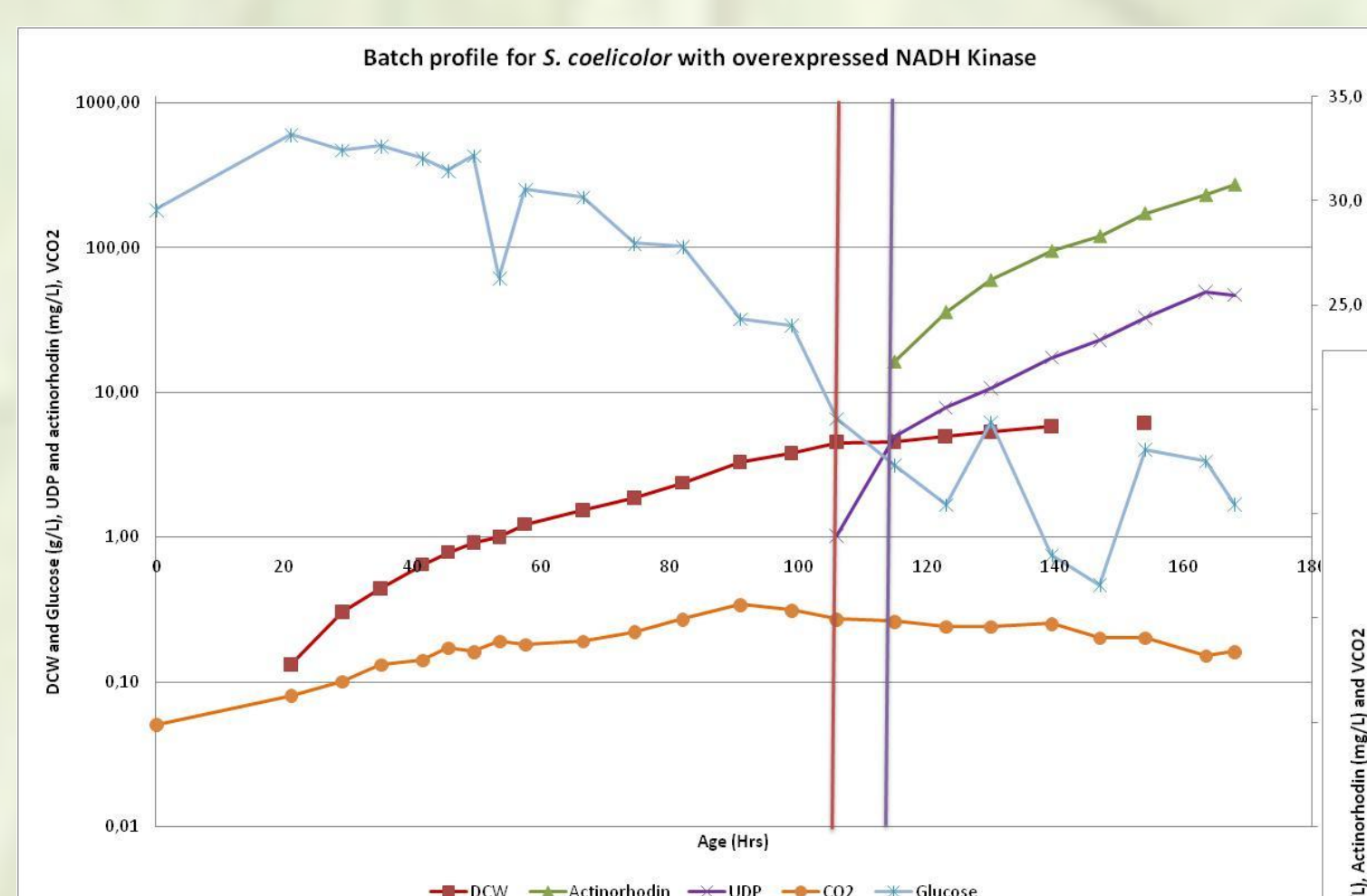


In vitro Tyrosine Phosphatase activity using PtkA



## Modification of redox levels

Antibiotic production is known to be induced when cell experiences stress. Hence, NADH oxidase (*Streptomyces pneumoniae*), NADH kinase (*Saccharomyces cerevisiae*) were expressed in *Streptomyces coelicolor* to study the effect of redox stress on antibiotic synthesis. The constructed strains carrying these enzymes have been assessed for physiological behavior.



## Synthetic Promoter Library

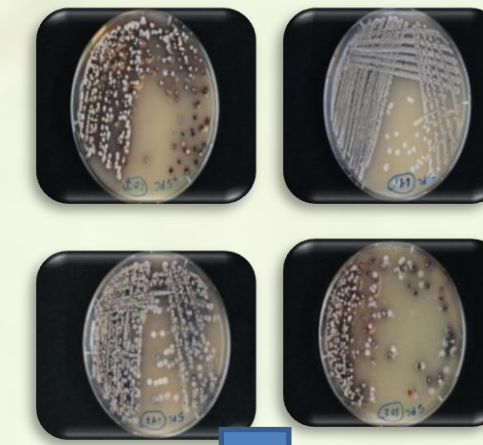
In the current study the native promoter of *actII orf4* was modified by randomizing the spacer sequence between the -35 box and -10 box and 5 nucleotide before and 5 nucleotides after -35 box and -10 box, respectively (see below). Furthermore, to ensure the stability of RNA the leader sequence was replaced with that of the glycolytic gene *pgi2*.

$-35$  TTGTGACGGCAAGCACATTGAAATCTGTTGAGTAGGCCTGTTATTGTCGGGG  $-10$   
~~CGAGGAGACGGGAGAATCTCGACGGGGGGGGCAG~~ATGAGATTCAACTTATTGGG  
ACGTG SD

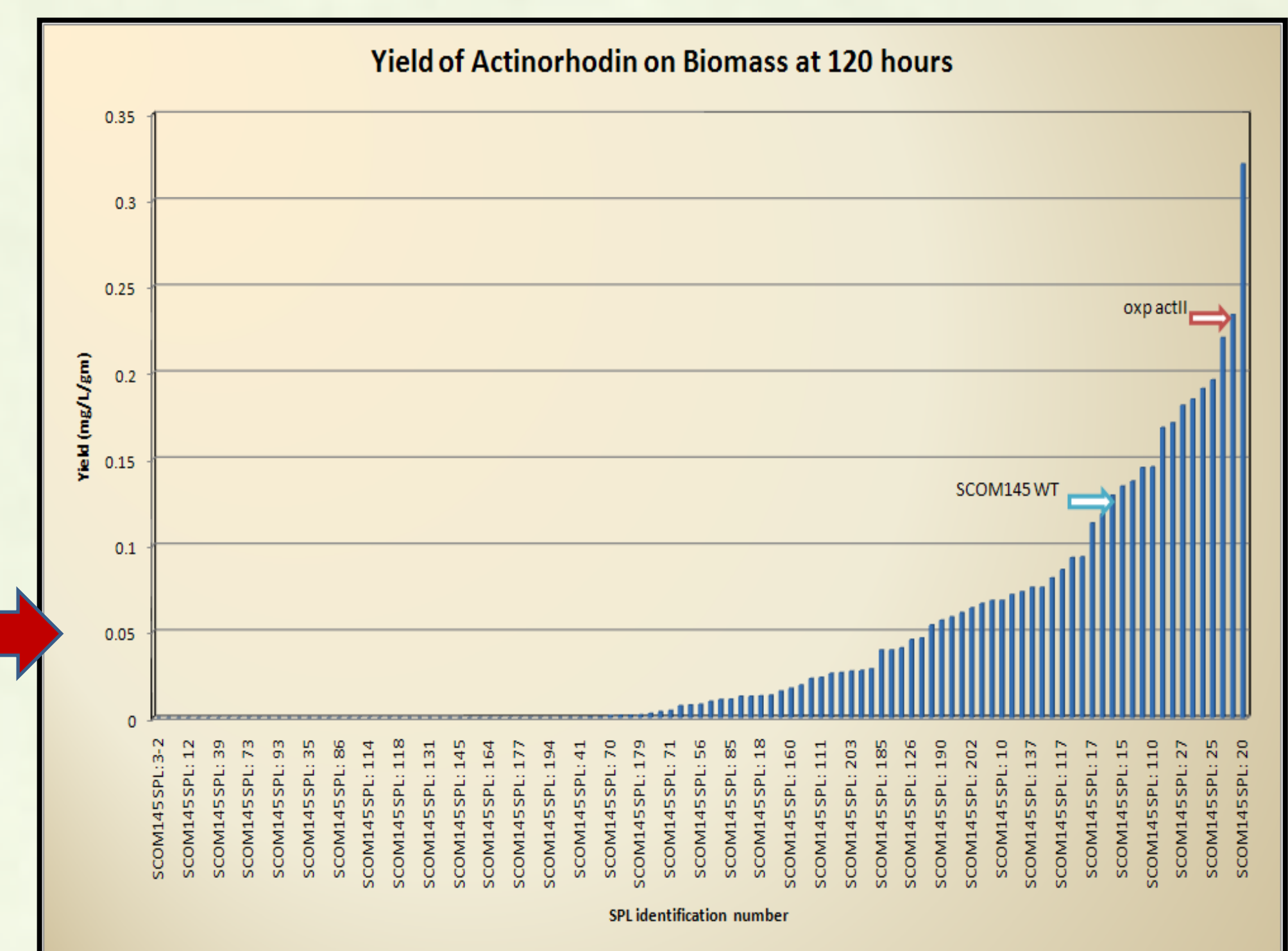
Native *actII ORF4*; The bases that are striked through correspond to the leader sequence that was replaced when generating the syntetic promoters, in blue in figure 3B.

RE site  $-35$  GTCATATGCTTGANNNNNTTGAANNNNNNNNNNNNNNNNNNNNNN  $-10$   
NNNNAGCTGTACAGGGGACAGCTGGGACACCCAAGGAAGAAGGCTGACGTC  
CGACATGAGATTCAACTTATTGGGACGTG SD  
Synthetic *actII ORF4*

Visual screening of ca 10.000 colonies



200 selected colonies further characterized



Detailed characterization of SPL20



Strain	$\mu_{exp}$ (h <sup>-1</sup> )	$\mu_{UDP}$ (h <sup>-1</sup> )	$\mu_{ACT}$ (h <sup>-1</sup> )	r P UDP (mg/L/hr)	r P ACT (mg/L/hr)	-rS (g/g)	Y P/X ACT (g/g)
Sco M145	0.1±0.01	0.06	-	1.5	15.1±1.4	1.8±0.01	0.13
Sco M145 oxp act II orf4	0.12	-	0.12	-	38.1	2.2±0.01	0.23
Sco SPL20	0.13	0.04	0.05	1.6±0.1	<b>70.9±1.5</b>	<b>2.5±0.01</b>	<b>0.32</b>

## ACKNOWLEDGEMENTS

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